seems to be no doubt that the weight loss observed is due to diminished food intake.

Although the salt wasting effects of amphetamine and d-amphetamine in the animal loaded with isotonic saline requires dosage levels in the rat far above those which would be considered therapeutic in man, we have found in the preliminary experiments quoted above that fasting human subjects show considerable natriuresis and diuresis with doses of 5.0-10.0 mg of d-amphetamine. The possibility that amphetamine and other sympathomimetic agents may be of value as adjuncts in the treatment of edema is being investigated.

Summary and conclusions. 1. Amphetamine and its isomers are diuretic and natriuretic in starved, normal and salt loaded rats. 2. The glomerular filtration rate of starved rats is elevated by d-amphetamine. The elevation of filtration rate is sufficient to account for the diuresis and natriuresis observed without postulating a decrease in tubular absorption of water or sodium.

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In vitro Inactivation of Newcastle Disease Virus by Penicillin Preparations. (20194)

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In the course of experiments in which it was attempted to estimate the concentration of Newcastle disease virus (NDV) by titration in embryonated eggs, a delay of the death of infected embryos was observed when the infecting virus suspension contained penicillin. Incubation of suspensions containing up to 10000 ELD_{50} (lethal for embryos) of the virus with 5000 units of penicillin for a few hours before injection into chick embryos resulted in an apparent 100-fold decrease in the infectivity of the virus. Streptomycin at concentrations up to 5 mg/ml had no detectable influence on the virus.

The present communication reports on the action of penicillin on NDV.

Materials and methods. Virus. The Newcastle disease virus used in these experiments was isolated from an epidemic in 1949† and stored in the form of infected allantoic fluid at −25°C for approximately 2 years. The virus killed susceptible chickens within 6 days after inoculation, and embryonated eggs within 72-96 hrs. In some experiments the live virus vaccine of Komarov and Goldsmit(9) was used. Titrations were carried out in 9-12 days embryonated eggs. Serial 10-fold dilutions of virus were prepared and 0.1 ml of each dilution was inoculated in the allantoic sacs of each of 4 to 6 eggs. Infected eggs were candled daily. Embryos dying within 24 hours of incubation were discarded. All eggs showing death of embryos after 24 hours were chilled for a few hours, opened, and their allantoic fluid tested for the presence of virus by means of a plate hemagglutination test (13). Virus titers were estimated according to the method of Reed and Muench(18). *Penicillin.* Crystalline penicillin G was used throughout (sodium salt—Merck, or potas-

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† Kindly supplied by Dr. Bornstein of the Government Veterinary Institute.

sium salt—Merck or Pfizer), showing an activity of 1500 to 1666 units per mg. The effectiveness of various means of inactivation of penicillin—such as heating, alkali, acid or penicillinase treatment—was also measured by means of dilution method against *Staphylococcus aureus*. *Penicillinase* (Wellcome) of the potency of 100000 penicillin neutralizing units per ml with 0.25% phenol preservative was used in certain experiments to inactivate the penicillin. Control tests showed that under the conditions of the experiment, neither the penicillinase (fresh as well as autoclaved) nor the phenol, exerted any measurable effect on the embryos or the virus.

Results. I. *Factors influencing action of penicillin on NDV.* A. *Time of incubation of penicillin—NDV mixtures.* Infected allantoic fluid containing 10^8-10^9 ELD_{50} per 0.1 ml was diluted 100-fold in sterile saline or phosphate buffer, in order to exclude possible interference by the concentrated allantoic fluid. The diluted virus (5 ml) was mixed with penicillin and incubated at 37°C for various periods of time. At the end of incubation period, the virus-penicillin mixture was titrated by inoculation of 10-fold dilutions into embryonated eggs. In this procedure the quantity of penicillin injected into the egg was less than 1000 units. At a concentration of 2000-5000 penicillin units per ml, the reduction of infectivity of the virus was not apparent within one hour of incubation, but became very pronounced by the third hour of incubation, by which time up to 10^5 ELD_{50} of the virus had been inactivated. As the concentration of penicillin was increased (up to 50000 units per ml) there was a corresponding decrease in the incubation time needed for significant inactivation of the virus. In the presence of concentrations higher than 100000 units per ml the virus was inactivated without prior incubation. It should be mentioned that the hemagglutinating titer of the virus was not affected by penicillin.

B. *Concentration of penicillin.* When NDV was incubated for 3 hours with penicillin at concentrations ranging from 200 to 20000 units per ml and the residual virus titrated by inoculation of serial tenfold dilutions of the mixture (in eggs), the degree of inactivation of the virus was found to be proportional to the concentration of the antibiotic (Fig. 1). At a concentration of 100-1000 units per ml the action of penicillin on the infectivity of the virus was indiscernible, but at 20000 units per ml a 10000-fold reduction of the virus concentration was observed. In several experiments 10000 units per ml inactivated completely 2×10^6 ELD_{50} of the virus.

II. *Activity of penicillin against NDV in vivo.* Serial 10-fold dilutions of NDV were prepared. From each dilution one ml was removed and mixed with one ml of penicillin solution containing 500000 units per ml. Two-tenths of the resulting mixtures were immediately injected into embryonated eggs and 10-day-old chicks. This procedure resulted in the protection of embryonated eggs and chicks against up to 4000 ELD_{50} of the virus. In the presence of higher concentrations of penicillin (800000 units per ml) even 10^6 ELD_{50} were not lethal to the embryos and no virus could be detected in them. The protection was, however, elicited only when the antibiotic and the virus were mixed before injection. Injection of penicillin prior to, or subsequent to the virus, protected neither chick embryos nor chicks. Simultaneous injection of virus and penicillin at different sites in chicks did not protect them against the disease. Prophylactic treatment by repeated injections of penicillin into birds exposed to an infected flock did not prevent them from contracting the disease at the same rate as non-treated chicks.

III. *Effect of various treatments on antiviral activity of penicillin. A. Activity of heat-treated penicillin.* When a solution of penicillin containing 10000 or 100000 units per ml was immersed for 10-15 minutes in a bath of boiling water, its antistaphylococcal activity fell to 1-100 units per ml. However, the infectivity of NDV incubated for 3 hours with this heated penicillin was reduced to the same extent, as when incubated with unheated penicillin (Table I). The virus was not affected whatsoever, when incubated with a quantity of fresh penicillin, which showed the same antistaphylococcal activity as that, which remained after heat inactivation.

B. *Activity of acid—or alkali-treated penicillin.* Five ml of a solution containing 50000

TABLE I. Comparison of Action of Fresh and Heated Penicillin on NDV.

NDV virus batch	Penicillin*	Penicillin conc., units/ml (×1000)	Incubation Time, hr	Incubation Temp., °C	Difference in virus conc. (log ELD_{50}) before and after incubation: Virus alone	Virus treated with: Fresh penicillin	Virus treated with: Heat inactivated penicillin
5	M-S	10	3	37	.0	3.5	2.0†
5	"	10	3	37		5.3‡	4.8
9	R-K	8	1†	45	.25	6.2‡	5.7†
			3	37			
9	"	8	3	37	.0	4.6	3.0
9	"	10	3	37	.7	2.1	4.4
10	"	10	3	37	.1	2.1	2.5‡

* M-S = Sodium G Penicillin (Merck) 1666 units/mg; R-K = Potassium G Penicillin (Rafa, Jerusalem) 1500 units/mg.

† Reduction of activity greater than indicated in table (titration endpoint not reached).

‡ *All* virus activity destroyed.

TABLE II. Comparison of Action of Penicillinase Treated and Untreated Penicillin on Newcastle Disease Virus.

Virus batch	Penicillin*	Penicillin conc., units/ml (×1000)	Virus conc. (log ELD_{50}): Virus alone	Virus + penicillin	Virus + penicillin + penicillinase	Reduction of virus activity by penicillin (log ELD_{50})	% inactivation of penicillin by penicillinase‡
A							
1	M-S	6	5.6	4.3	5.5	1.3	
5	"	10	5.0	2.5	3.5	2.5	90
9§	R-K	8	6.6	5.4	6.3	1.2	87
9§	"	8	6.6	2.0	6.0	4.6	99.99
9	"	10	6.6	4.5	5.3	2.0	84
10	"	10	2.5	.37	.5	2.13	33
B							
9	HR-K	8	6.6	3.5	5.8	3.0	99.4
9	"	10	6.6	2.3	4.6	4.3	99.5
10	"	10	2.5	0 †	1.7	2.5	98
C							
13	M-S	20	5.3	3.3	4.1	2.0	84
					5.1‖		98.4
14	"	20	5.4	.5	3.1	4.9	99.76
					5.2‖		100
13	"	20	5.0	0	0	5.0†	0
					5.5‖		100

* and † as in Table I. HR-K = Heat-inactivated potassium G penicillin.

‡ $\left(100 - \frac{\text{antilog column VI} \times 100}{\text{antilog column VII}}\right)$ %.

§ Data represent results after incubation of virus + penicillin mixture 1.5 and 3 hr respectively.

‖ Penicillin incubated with *autoclaved* penicillinase 30 min. before addition of virus; additional incubation 3 hr.

units of penicillin in distilled water or saline were treated wth 0.1 ml of N/1 hydrochloric acid or with 0.1 ml of N/1 sodium hydroxide and incubated for 30-60 minutes at 56°C. The acid treatment presumably brings about transformation of penicillin to penillic and penilloic acid, whereas alkali causes the formation of penicillic acid in a manner similar to the action of penicillinase(4). At the end of the incubation period the solutions were neutralized and incubated with NDV for 3 hrs at 37°C. Both alkali and acid treatment reduced the antistaphylococcal potency of penicillin 100-1000-fold, but whereas the acid treated penicillin lost completely its virucidal property (irrespective of its antistaphylococ-

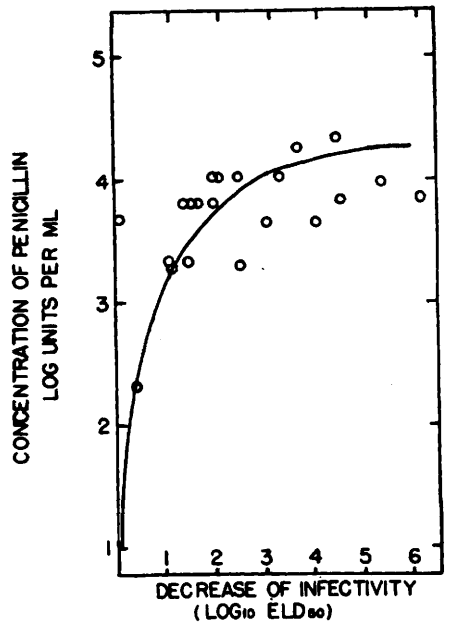

FIG. 1. Reduction of infectivity of NDV by penicillin (incubation 3 hr).

cal potency), the antiviral activity of the alkali treated antibiotic was reduced at the same rate as the antistaphylococcal activity.

C. *Influence of penicillinase on the antiviral activity of penicillin.* When penicillinase‡ at adequate concentration was mixed with penicillin *prior to* incubation of the latter with the virus, the inactivation of the virus was prevented (Table 2A). Penicillinase also inhibited the antiviral activity of heat treated penicillin (Table IIB). Later experiments showed that autoclaved, *i.e.* enzymatically inactive penicillinase also neutralized the antiviral activity of fresh penicillin (Table IIC). When penicillinase was added to the mixture of virus and penicillin *at the end* of the incubation period, no reversal of virus inactivation took place.

IV. *Activity of penicillin against other viruses.* Preliminary experiments showed that penicillin was active against the viruses of influenza (PR8) and Eastern Equine Encephalitis (EEE). The incubation of these viruses in 5 ml saline with 50000 units per ml of penicillin G for 3 hours resulted in a 10000-fold reduction of influenza activity and complete destruction of 10^8 LD_{50} of EEE.

Discussion. It is generally assumed that penicillin does not affect microorganisms which have no measurable activity outside the host cell. Even the large viruses like Rickettsiae and members of the Psittacosis-Lymphogranuloma group that are inactivated *in vivo* (embryonated eggs and mice) by relatively low doses of penicillin, are affected *in vitro* only by very high concentrations (40000-100000 units/ml) of this antibiotic(5,7,16). Eaton *et al.*(2) and Hamre and Rake(7) present evidence that the effect of penicillin *in vitro* on agents of lymphogranuloma venereum, feline and murine pneumonitis might have been due to impurities. It was also found by Groupé and Rake(6) that a certain batch of commercial penicillin, with a potency of 988 units/mg, destroyed the viruses of canary pox, smallpox and vaccinia after contact of 2 hours. Inactivation by clarase or by boiling did not affect the antiviral property of this penicillin; crystalline penicillin G (25000 units) alone was inactive against these viruses. The effect was apparently due to impurities. The results obtained by us with crystalline penicillin G indicate that we are not dealing with the same active entity as Groupé and Rake, though the possibility that impurities may be responsible for the findings cannot be ruled out. Penicillin has been reported to be inactive against the viruses of vaccinia(2,8,16), fowl-pox(20), rabies(11,19), herpes and influenza(21), mumps(1), eastern equine and St. Louis encephalitis(16), coxsackie(14), sarcoma(11), bacterial viruses(15,17) and plant viruses(13). In most of these investigations (1,8,10,11,16,17,20,21), however, the concentration of penicillin was comparatively low and/or no incubation *in vitro* with the virus was attempted.

The phenomenon of inactivation of NDV by penicillin is surprising, for this virus is a member of a group which was hitherto thought to be refractory to penicillin. The lack of therapeutic activity of penicillin against NDV *in vivo* (II) may perhaps be explained as being due to insufficient concentrations of the antibiotic attained in the chick embryos or chicks. The maximal concentration of peni-

‡ The term penicillinase refers to the commercial preparation described in *Methods*.

cillin in the egg or chick could not have exceeded 1000 units/ml, which was found to be insufficient for the inactivation of the virus *in vitro*.

The inability of the penicillinase preparation to restore the virus to its original activity, when added to the penicillin-virus mixture at the end of the incubation period, indicates an irreversible inactivation of the virus. Whilst acid or alkali treatment destroys both the antiviral and the antistaphylococcal properties of penicillin, boiling or treatment with autoclaved penicillinase affect these properties in a different manner: Heat treated penicillin has lost its antistaphylococcal activity, but is still fully active against the virus; a heat-stable, non-enzymatic factor present in the penicillinase abolishes the antiviral, but not the antibacterial activity of penicillin.

The problems which remain to be elucidated are: 1) whether the antiviral properties of "penicillin" are due to a) some thermostable, functional group of the penicillin molecule itself, or b) a highly active, contaminating substance present in crystalline penicillin G which is heat stable but sensitive to acid and alkali; 2) what is the heat-stable substance in penicillinase which destroys the antiviral activity of penicillin.

Summary. 1. When Newcastle disease virus is mixed with penicillin, inactivation of the virus takes place. The extent of the inactivation depends both on the concentration of penicillin and the time of incubation of the mixture. Incubation of the virus *in vitro* for 3 hours with more than 5000 units per ml of crystalline penicillin G results in at least a 100-fold reduction of the infectivity of the virus and up to 2 million ELD_{50} of the virus may be completely inactivated. 2. The virus inactivating property of penicillin may be destroyed by acid or alkali treatment but is not affected by heating. Penicillinase also inactivates the antiviral properties of fresh and heated penicillin. However, this effect is apparently not due to enzymic action. Virus *previously* inactivated by fresh or heated penicillin cannot be restored to its original potency when penicillin is neutralized by penicillinase. 3. Injection of 50000 units of penicillin *mixed* with the virus protects chick embryos and 10-day-old chicks against as many as 4000 ELD_{50} of the virus. At this concentration, however, penicillin has no prophylactic or therapeutic activity.

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